Boll Weevil (Coleoptera: Curculionidae) Bait Sticks: Toxicity and Malathion Content

ERIC J. VILLAVASO, JOSEPH E. MULROONEY,[1] AND WILLIAM L. MCGOVERN

USDA–ARS Southern Insect Management Research Unit, Mississippi State, Mississippi 39762–5367

J. Econ. Entomol. 96(2): 311–321 (2003)

ABSTRACT Assays of malathion content and toxicity to boll weevil, *Anthonomus grandis grandis* Boheman, were conducted on boll weevil bait sticks, now marketed as Boll Weevil Attract and Control Tubes (BWACTs; Plato Industries, Houston, TX). In general, the longer BWACTs were in the field, the lower the mortality of weevils that were exposed to them. Bioassays of weevil mortality correlated with hexane washes of BWACT surfaces showed highly variable mortality when surface malathion fell below ≈20 ng per 1 μl of hexane, but consistently high mortality (≥90%) when surface malathion was above 30 ng per 1 μl of hexane. A linear equation was calculated to predict mortality as a function of malathion on a BWACT surface. Although mortality was related to surface amounts of malathion, it was unrelated to the total amount of malathion present in BWACTs. Similarly, surface malathion was unrelated to the total amount present in BWACTs. As with mortality, amount of surface malathion declined with time, but total malathion did not decline with time. Boll weevils placed on fresh BWACTs tended to accumulate more malathion and died in greater numbers as time spent on fresh tubes increased, but not as time spent on tubes aged in the field (for 5 mo total) increased. Weevils that landed on tubes after a short flight died in approximately the same numbers as those that were placed on tubes using proper methodology. The amount of malathion expected to cause 90% mortality of boll weevils subjected to proper methodology was 47% higher than for a less stringent methodology (34.3 versus 23.4 ng), which demonstrates the importance of strictly adhering to proper methodology; nevertheless, chemical assay of malathion on the BWACT surface proved to be a more consistent measure of BWACT toxicity than bioassay, and it should replace the bioassay.

KEY WORDS boll weevil, bait, malathion, toxicity

AN ATTRACT-AND-KILL device for boll weevil, *Anthonomus grandis grandis* Boheman, was developed by McKibben et al. (1990). This device, the boll weevil bait stick, consisted of a vertically oriented wooden broomstick coated with a formulation, the major ingredients of which were grandlure (a synthetic form of the boll weevil's aggregation and sex pheromone; Tumlinson et al. 1969), cottonseed oil (a feeding stimulant), and an insecticide (cyfluthrin). Plato Industries (Houston, TX) is the sole commercial provider of bait sticks, now marketed as Boll Weevil Attract and Control Tubes (BWACTs; Plato Industries, Houston, TX). BWACTs are similar to the original bait sticks except they are hollow, cardboard-like tubes and malathion is the toxicant. According to the BWACT label, malathion comprises 37.27% of the BWACT coating. Reports of the effectiveness of the original bait sticks and BWACTs have been both favorable (McKibben et al. 1991, 1994; Smith et al. 1992, 1994, McGovern et al. 1993, 1995, 1996; Daxl et al. 1995; Parvin 1995; Langston 1995, 1996; Villavaso et al. 1998) and unfavorable (Fuchs and Minzenmayer 1992; Karner and Goodson 1993, 1995; Parker et al. 1995; Spurgeon et al. 1999).

Mortality of boll weevils exposed to bait tubes under field conditions for various times has not been consistent (EJV, unpublished data). Differences in environmental conditions sustained by the tubes probably play a role in their toxicity and the persistence of the toxicity. One of the objectives of this study was to determine whether a relationship exists between BWACT toxicity to boll weevils as determined by bioassay and the amount of malathion in or on BWACTs as determined by chemical assay. This knowledge would allow for an estimation of bait stick toxicity at any given time without the need for maintaining a colony of boll weevils, and quality control standards could be formulated from the data. A second objective was to determine whether a relationship exists between the length of time a boll weevil spends on a BWACT and the amount of malathion adhering to the weevil and subsequent mortality.

Most research on BWACT toxicity has been conducted by placing boll weevils on tubes (methodology of Villavaso et al. 1998; designated forced-contact assay, Spurgeon et al. 1999) rather than by allowing them

[1] Current address: U. S. Forest Service, 201 Lincoln Green, Starkville, MS, 39759 (e-mail: EVillavaso@msa-msstate.ars.usda.gov).

to land or crawl on the tubes naturally. Substantially higher mortality was recorded for weevils subjected to forced-contact assay compared with assays in which weevils landed on tubes naturally or crawled onto them after having been placed on the untreated portion of the tube (Spurgeon et al. 1999, Spurgeon 2001). The adequacy of both the forced-contact assay and ability of BWACTs to kill weevils naturally introduced to them has been questioned (Spurgeon 2001). A third objective of this study was to establish whether the forced-contact assay is a reasonable method to determine toxicity of bait tubes.

Materials and Methods

Bait tubes were supplied by Plato Industries and the Southeastern Boll Weevil Eradication Foundation, Montgomery, AL, (source also Plato Industries). Boll weevils were from a long-established laboratory colony at Mississippi State, MS; native males had been captured and added to the colony every one to 2 yr.

Toxicity of BWACTs over Time. Although we took measurements of BWACT toxicity as time in the field increased, the major purpose of allowing the BWACTs to age in the field was to determine the relationship between a chemical assay of the amount of malathion in and on a BWACT and a bioassay of boll weevil mortality. A greater range of malathion amounts and corresponding boll weevil mortalities could be attained as BWACTs aged in the field.

Standard BWACTs from three boxes manufactured on different dates (box 1, manufactured in 1999, about the same time as boxes two and 3; box label was illegible when received, so the exact date is unknown; box 2, 2 March 1999; box 3, 1 June 1999) were bioassayed. Boxes two and three were taken from the same lots as hundreds of boxes used in the Mississippi boll weevil eradication program in 1999. Five tubes from each box were taken into the laboratory for bioassay on the day the box was opened. Also on that day, an additional 35 tubes from each box were lined up 0.5 m apart in a grassy plot outside of the laboratory. Tubes were kept vertical and stable by driving the cylindrical wooden stakes (46 by 1.9 cm) provided with each box of BWACTs into the ground and sliding the BWACTs over them. Once a week for 6 wk after the initial bioassay, five tubes from each box were removed from the grassy plot and taken into the laboratory for bioassay. Bioassays were conducted by placing 10 boll weevils, one at a time, on each tube and allowing each weevil to remain there for 30 s (Villavaso et al. 1998; see proper methodology below). Then they were put into 60- by 15-mm (height) plastic petri dishes, one weevil per dish, and mortality was recorded 24 h later. Ten weevils not placed on BWACTs were used as controls. Tubes from box one also were used in a preliminary test to decide whether one or two hexane washes would be necessary for measuring the amount of malathion on the surface of BWACTs.

Relationship between Bioassay Type and Chemical Assay: Proper versus Improper Forced-Contact. The forced-contact method, properly conducted, is exacting and time-consuming. Weevils must be on their feet, and motionless or crawling slowly when grasped firmly with fine forceps at a point approximately two-thirds of the distance behind the anterior portion of the elytra. Picking up a weevil this way often causes it to extend its legs upward and outward, putting it immediately into position for placing it on the BWACT with only its tarsi, tarsal claws, and adjacent leg structures contacting the tube. Weevils offered a tube in such a manner tend to grasp it and adapt to it as if they had landed there naturally. Weevils not assuming the open-legs position can generally be induced to assume that position by gently touching the distal portions of their legs to a piece of paper or similar material while they are being held with the forceps. Softer materials such as cotton cloth generally do not work because weevils are able to cling tightly to them and free themselves from the grip of the forceps. For proper forced-contact, weevils that cannot be induced to assume the open-leg position must not be placed on BWACTs.

Weevils were placed on the tubes by two methods:1) the proper forced-contact method, executed exactly as described above; and 2) an improper forced-contact method executed exactly as in method 1 except that weevils were placed on tubes in a less diligent manner with parts other than their tarsi, tarsal claws, and adjacent leg structures being allowed to contact the tube. Ten weevils, one at a time, were placed on the surface of each BWACT and allowed to remain for 30 s. The BWACT was then gently tapped so that the weevil would fall from it onto a clean surface from which it was transferred with forceps into a 15- by 60-mm plastic petri dish. Ten weevils not placed on BWACTs were used as controls. Mortality was recorded 24 h later.

Extracting Malathion from BWACTs. Within 2 h after the bioassay described above, three 7.5 cm sections were cut from each tube between 10.2 and 17.8, 30.5 and 38.1, and 50.8 and 58.4 cm from the top of the tube. We analyzed these sections to estimate the average amount of malathion on the surface of a tube so that we could compare that amount to mortality of weevils exposed to that tube and to total amount of malathion in and on a tube. Rubber stoppers were put into the ends of each section of tube to avoid washing malathion from the tube's inner surface. The inner surface also is coated with malathion, but it is not subjected to the same weathering as the outer surface, and weevils seldom contact it. After a section of tube was stoppered, 10 ml of hexane was pipetted over its surface as it was rotated horizontally by hand over a glass funnel directed into a 20-ml scintillation vial. In a preliminary test with BWACTs from box 1, a second 10-ml portion of hexane was pipetted over each section of tube immediately after the first to estimate the extent of the first surface wash and to allow us to decide whether a second wash was constructive. The second wash was conducted for weeks 2–8 only. Vials were labeled so that the bioassay and the chemical assay could be easily correlated. Results of the preliminary test (see Extracting Malathion from

BWACTs) indicated no need for the second application of 10 ml of hexane, so only one 10-ml application was used for tests with boxes two and 3.

Estimates of the total amount of malathion in BWACTs also were made. After the hexane wash was completed, the top 7.5 cm section of each tube was placed into a jar containing 200 ml of hexane. Each jar was then sealed with a screw top lid, and each section sunk to the bottom of the jar. Sections were allowed to soak with occasional agitation for 7 d. Then a 10-ml portion of the mix was pipetted into a 20-ml scintillation vial and labeled for chemical assay. Additionally, the amount of malathion measured from the top sections after the 7-d soaking was compared with the amount that had been washed from the surface of the top 7.5 cm sections immediately before.

Aliquots (2 ml) of the hexane-malathion mixes were placed in autosampler vials for analysis by gas chromatography. A Hewlett–Packard 5890 gas chromatograph equipped with a flame photometric detector, an autosampler, and ChemStation (Hewlett–Packard, Palo Alto, CA) software was used to quantitate malathion residues.

The parameters of our residue analyses method were as follows: injector temperature, 200°C; oven program, 120°C initial temperature with a 25°C/min increase to 250°C for 1 min, and then a 25°C/min increase to 280°C for 4 min. A Hewlett–Packard Ultra-1 cross-linked methyl silicone gum phase column (25 m by 0.32 mm by 0.52 μm) with a 2.65-ml/min flow of helium was used. Retention time of malathion was 5.597 min. Malathion content was calculated as nanograms of malathion per microliter of hexane. All plots, regression equations, and r^2 values were produced with SigmaPlot 5.0 (SPSS, Chicago, IL).

Landing versus Forced-Contact. Two tests of landing versus forced-contact were conducted. For the first test, assays were performed on the day the BWACTs were removed from the shipping box, and again on the following day. Between tests BWACTs were placed in a row outside the greenhouse to age them, i.e., to expose them to environmental conditions that tend to reduce their potency over time. Thus, we could determine whether aged BWACTs remained toxic to weevils landing on them naturally. Seven BWACTs were taken from the row to the greenhouse and arranged in a circle ≈40 cm in diameter. Ten to 15 weevils at a time were placed in a topless 237-ml cylindrical ice cream carton, the walls of which had been cut to resemble a crown. The points of the crown were apices from which a weevil could easily fly, and the carton containing the weevils was elevated ≈12 cm from the greenhouse floor. When a weevil flew from the carton and landed on a tube, it was visually observed until it flew or fell from the tube. A stopwatch was used to determine the time each weevil spent on a tube. Weevils leaving the tubes were captured and placed in 15- by 60-mm plastic petri dishes, one weevil per dish. The time a weevil spent on a tube was recorded on the dish. Samples of weevils flying from the carton and not landing on tubes were used as controls. Weevils from the same group as those used in the greenhouse were tested by the forced-contact method. After exposure to a tube, each weevil was placed in a 15- by 60-mm plastic petri dish, and mortality was recorded 24 h later.

The second test was conducted using the same methods of bioassay described above on a different group of BWACTs and boll weevils. For this test, tubes were assayed on the day they were removed from the shipping box and at 7-d intervals for 28 d.

Malathion Adherence to Individual Boll Weevils. Amount and Subsequent Mortality. BWACTs from a fourth box (31 August 1999) stored in a closed shed and BWACTs from the same box, but which had been in the field two 9 September9–20 January 2000, were bioassayed using proper methodology. Groups of 20 boll weevils, one at a time, were placed on either the fresh or aged tubes for 0-, 5-, and 10-s intervals and at sequential 10-s intervals up to 100 s. To determine mortality, 10 weevils from each group were put into 15- by 60-mm plastic petri dishes, one per dish, and mortality was recorded 24 h later. To determine malathion adherence to individual boll weevils, each of the other 10 weevils was dropped into a 2-ml autosampler vial to which 1 ml of hexane was added. Chemical analyses were conducted as described above.

Results and Discussion

Toxicity of BWACTs over Time. Weevil mortality observed over three different groups of BWACTs tested at different times can be seen in Fig. 1. At least 90% of boll weevils died within 24 h of being placed on BWACTs for the first two assays. Mortality decreased over time for boxes one and 2, but followed an unusual pattern for box 3. Weevils exposed to tubes from box three followed a pattern similar to that of boxes one and two for the first three bioassays; a steep decrease in mortality followed by an equally steep increase in mortality occurred between the third, fourth, and fifth assays, and then mortality rose through the seventh bioassay. We have no explanation for this odd occurrence. Mortality observed on all five BWACT each week was reasonably consistent, and a mix-up in the data collection process has been ruled out. Maintenance of 100% or near 100% mortality previously reported at six locations over a 6-wk period was not duplicated herein (Villavaso et al. 1998). No mortality was observed in control weevils.

Relationship between Bioassay and Chemical Assay: Proper versus Improper Forced-Contact. For both proper and improper forced-contact, BWACTs with ≤20 ng malathion per 1 μl of hexane produced highly variable mortality of boll weevils exposed to them; thus, bioassays resulting in high mortality are not necessarily associated with high levels of malathion (Fig. 2). Conversely, BWACTs with >30 ng malathion per 1 μl of hexane washed from the tube surface produced consistently high mortality (≥90%); thus, high levels of surface malathion (>30 ng per 1 μl of hexane) are closely associated with high levels of mortality.

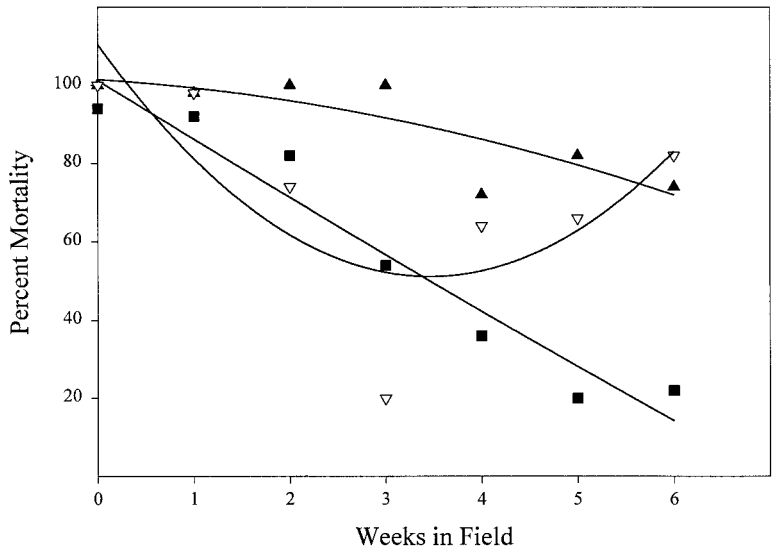

Fig. 1. Mortality of boll weevils 24 h after being placed on BWACTs for 30 s. Squares, box 1; triangles, box 2; inverted triangles, box 3. BWACTs from different boxes were manufactured on different dates.

Lines depicting malathion content versus percentage of mortality for both types of contact were calculated using an envelope curve over 11 data points. The points for 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90% mortality were those that were associated with the greatest amount of malathion producing the mortality, and the point for 100% mortality was the lowest amount at which 100% mortality always occurred (Fig. 2). Equations for the lines are as follows:

$$f = -20.9 + 3.2x \text{ (proper forced-contact; } r^2 = 0.85) \quad [1]$$

$$f = -37.2 + 5.4x \text{ (improper forced-contact; } r^2 = 0.57) \quad [2]$$

where x is average amount of malathion found in 10-ml washes of the top, middle, and bottom 7.5 cm sections of BWACT in nanograms per microliter of hexane. These equations estimate the least amount of mortality expected for any given dose of malathion, and they indicate that the amount of mortality associated with any given dose of malathion is higher for proper than improper forced-contact. Based on these equations, the amount of malathion expected to cause 90% mor-

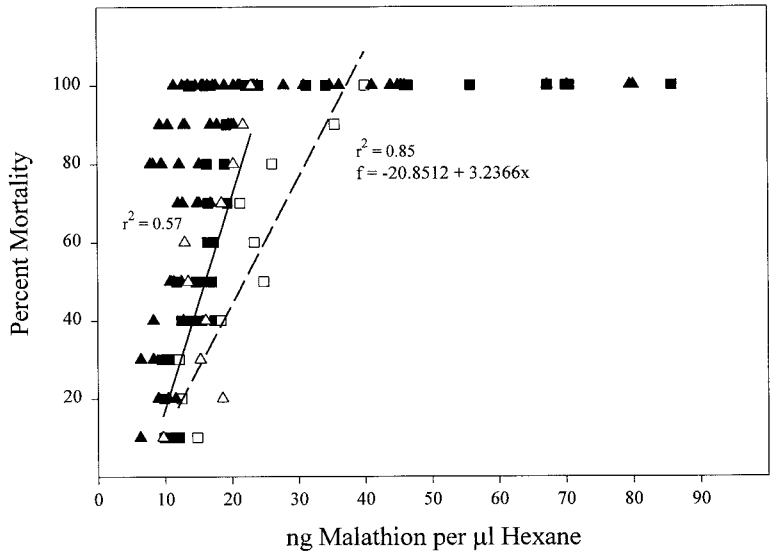

Fig. 2. Relationship between amount of malathion washed from the surface of BWACTs with hexane and mortality of boll weevils subjected to proper (squares) and improper (triangles) methodology. Open symbols used for regression lines.

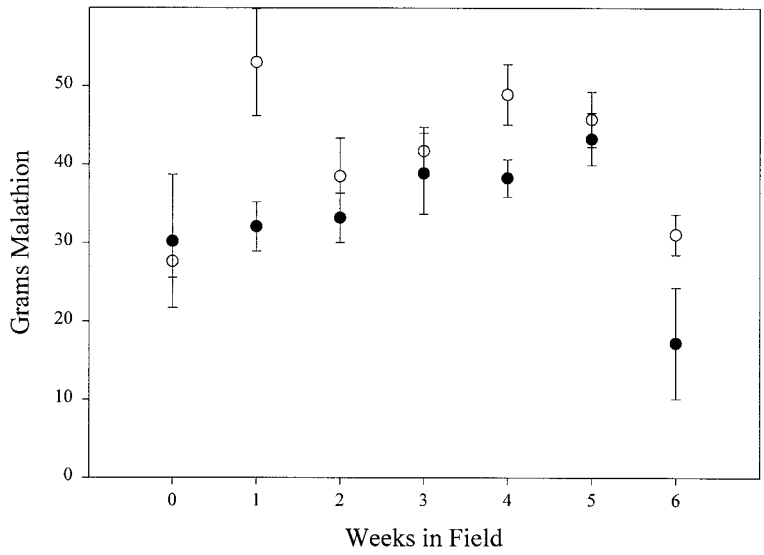

Fig. 3. Total amount (with standard deviation) of malathion extracted from BWACTs with hexane over a 6-wk period. Open circles, box 2; solid circles, box 3. BWACTs from different boxes were manufactured on different dates.

tality of boll weevils subjected to properly conducted forced-contact is 47% higher than that seen for improper forced-contact (34.3 versus 23.4 ng), which demonstrates the importance of strictly adhering to proper methodology for the bioassay. The proper forced-contact equation can be used to estimate the potency of a group of BWACTs at any time, and it is being used by the Southeastern Boll Weevil Eradication Foundation, Montgomery, AL, as the quality standard for BWACTs.

Extracting Malathion from BWACTs. Over a 6-wk period in the field, the total amount of malathion remaining in BWACT from boxes two and three was estimated (from analyzing malathion extracted from the top section of tubes after they had soaked in hexane for 7 d) to be between 30 and 50 g and averaged 37.1 ± 9.1 g (SD) per tube (Fig. 3). Total malathion in and on the tubes did not decline with increased time in the field. Additionally, there was no apparent correlation between the total amount of malathion extracted from a 7.5-cm section of BWACT (7-d hexane soak) and the amount present on the surface of that BWACT section (10-ml hexane wash; Fig. 4). Factors other than total amount of malathion apparently control the amount reaching the tube surface. Based on equation 1 above, recovery of 34.3 ng of malathion per l μl of hexane applied to the outer surface of a 7.5-cm-long section of a tube will produce 90% mortality of boll weevils exposed to that tube for 30 s. Converting this amount to total malathion on the surface of a BWACT (0.00400 g) and dividing by the total amount of malathion in and on the BWACT (37.1 g) indicates that 0.01% of the total malathion in a BWACT must be present on the tube surface to produce 90% mortality.

Results of the test with box one tubes used as a preliminary test to determine the need for one or two consecutive 10-ml washes showed similar declines in malathion content among top, middle, and bottom sections for first washes over the test period (Fig. 5). Such declines did not occur for second washes. No matter how much malathion was found in the first 10-ml wash, the second wash always contained ≈4 ng of malathion per 1 μl of hexane (40 μg per 10 ml). This finding suggests that the first wash was removing a surface layer containing a highly variable amount of malathion and the second was removing a somewhat deeper, more consistent amount that was perhaps in a transitional area between the tube surface and the abundant supply of malathion probably absorbed by the tube material (McKibben et al. 1993). Most of the malathion in a tube remained below the layer that could be washed from the tube surface. From these results, we concluded that one 10-ml hexane wash was sufficient for determining the amount of surface malathion to compare with weevil mortality.

The amount of malathion washed from the surface of fresh BWACTs from boxes two and three ranged between 44 and 56 ng/μl of hexane (Fig. 6). By the end of the first week in the field, malathion amounts had decreased to ≈30 ng and then tended to level near or below 20 ng for weeks 2–7. As we might expect from the data presented in Fig. 2, consistently high mortality in weevils exposed to BWACTs from boxes two and three occurred during weeks 0 and one when malathion amounts were > 30 ng per 1 μl hexane, but considerable variability in mortality occurred during weeks 2–7 when malathion amounts were near 20 ng (Fig. 1). Taken together, the data presented in Figs. 1 and 2, and six strongly suggest that BWACTs should be replaced with fresh tubes if surface malathion falls below 30 ng and demonstrate the importance of replacing the bioassay with the chemical assay.

Landing versus Forced-Contact. Mortality of weevils landing on BWACTs or placed on them (forced-

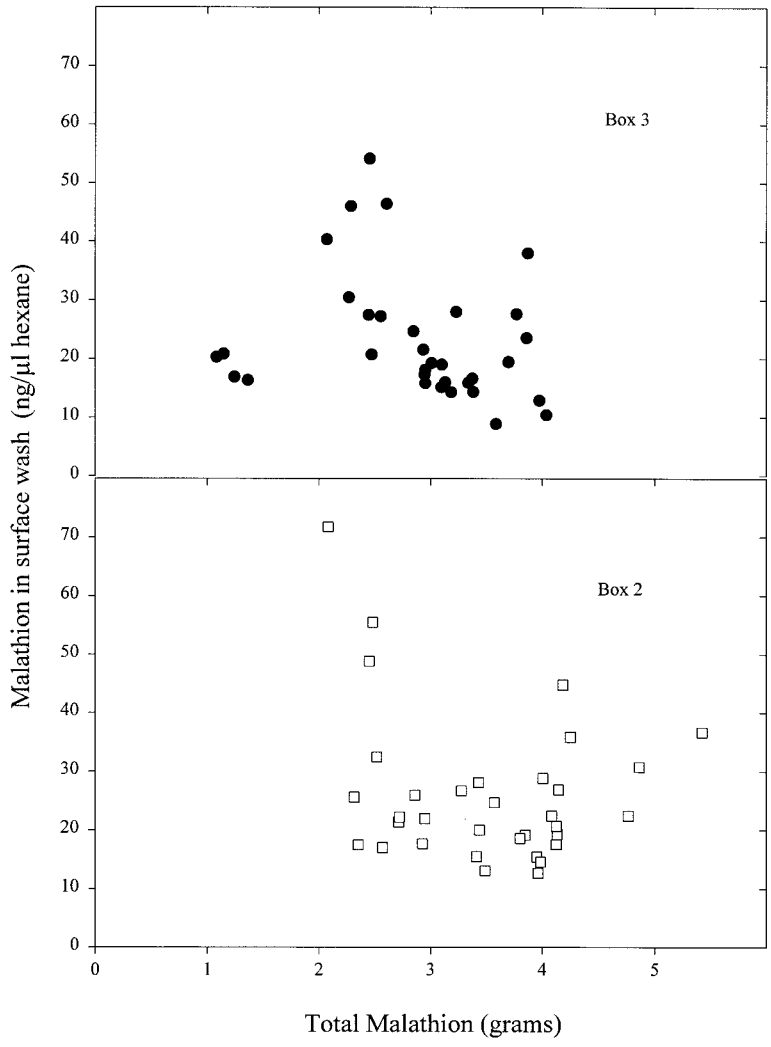

Fig. 4. Total amount of malathion (grams) extracted from top 7. 5 cm section of BWACTs versus amount (ng/μl hexane) from surface wash of same section.

contact) was similar (Table 1). For the landing treatment, the weevils themselves determined the time interval that they remained on the BWACTs, which resulted in many time periods with a small sample size for each period. Therefore, we pooled time intervals for the landing treatment. Forced-contact assay has previously been reported for discrete selected periods of time, usually 30 s, and we selected both 15- and 30-s periods for the forced-contact treatment.

Mortality for 1–15 s for the landing treatment was somewhat lower than that of the 15-s forced-contact treatment (Table 1); however, many weevils in the 1- to 15-s treatment were on a BWACT for less than the full 15-s period allotted to all forced-contact weevils, so somewhat lower mortality could be expected. Likewise, mortality for the 15- to 30-s group included many weevils on a BWACT $<$ 30 s, whereas all 30-s forced-contact weevils remained on a BWACT for a full 30 s. No control weevils died.

The data in Table 1 indicate that when appropriate levels of malathion are present on the BWACT surface, weevil exposure either by landing or forced-contact will result in high mortality. We have made numerous field observations of boll weevils landing on BWACTs, falling off within seconds or minutes, and dying soon afterwards on the ground or in containers placed at ground level to collect fallen weevils. As malathion levels on the BWACT surface decrease, percentage of mortality for both landing and forced-contact decreases.

Malathion Adherence to Individual Boll Weevils: Amount and Subsequent Mortality. Mortality of weevils placed on fresh BWACTs taken from a box in storage for ≈5 mo increased sharply with time of exposure, peaked between 20 and 30 s, and can be expressed by the sigmoidal three-parameter curve (Fig. 7). A minimum mortality of 30% occurred at the 5-s time interval, increased to 80% at the 10- and 20-s

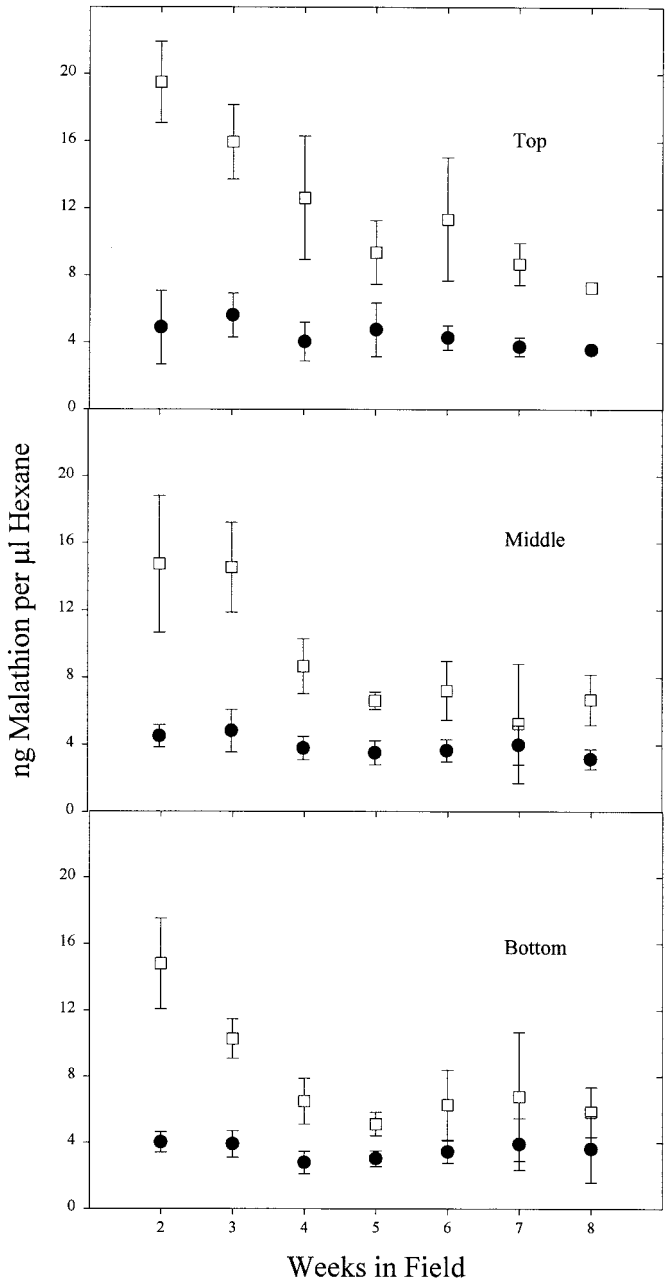

Fig. 5. Amount (with standard deviation) of malathion removed from the surface of BWACTs by two washes of 10 ml of hexane. Squares, first wash; circles, second wash.

intervals, and reached 100% at the 30-s interval. Except for the 50-s interval, mortality was 100% for all eight intervals after 20 s. No control weevils died. In contrast, mortality of weevils placed on aged BWACTs (from same box as fresh, but in the field for ≈5 mo) did not exceed 30%, and there was no trend toward increased mortality as time spent on BWACTs increased (Fig. 7). Malathion on aged BWACT surfaces may be limited to a few small, randomly occurring spots that weevils contact by chance.

The sigmoidal three-parameter curve for amount of malathion adhering to individual weevils placed on the fresh BWACTs versus percentage of mortality (Fig. 8) was almost identical to the curve for percentage of mortality versus time (Fig. 7). Mortality rose sharply between the 1- and 2-μg marks and was 100% at seven of the eight points > 1.5 μg.

In agreement with increased mortality being associated with increased time spent on a fresh BWACTs, the amount of malathion adhering to a boll weevil

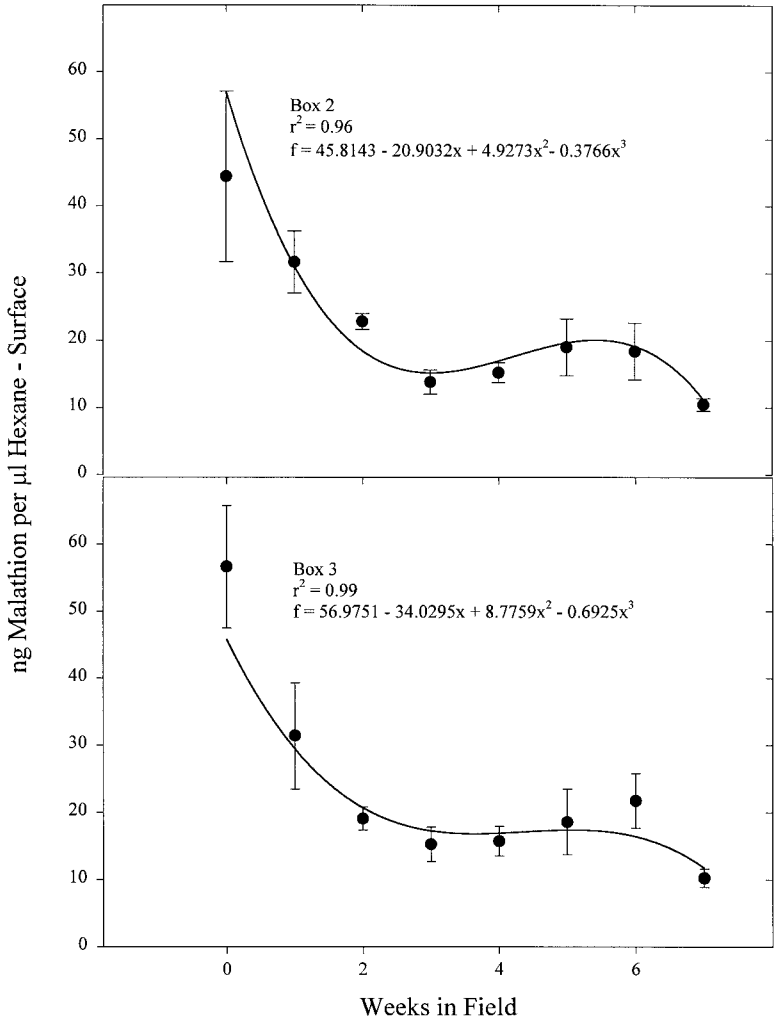

Fig. 6. Amount (with standard deviation) of malathion removed from the surface of BWACTs over a 6-wk period.

increased with time spent on a fresh BWACT (Fig. 9). In contrast with the sigmoidal relationships depicted in Figs. 7 and 8, we saw a strong linear increase in malathion per weevil with increased time of exposure. Mulrooney (2001) reported results that correspond favorably with those reported herein: malathion accumulation was greater on boll weevils that traveled greater distances over treated cotton leaves, and mortality increased as malathion accumulation increased.

According to the equation presented in Fig. 9, initial contact of a weevil to a BWACT resulted in 0.56 μg of malathion adhering to it. Incorporating this amount

Table 1. Mortality of boll weevils landing versus forced-contact on Boll Weevil Attract and Control Tubes

Week	Seconds on BWACTs					
	Landing				Forced Contact	
	1	>1–15	>15–30	>30	15	30
0[a]	11/15 (73)	30/30 (100)	5/5 (100)	3/3 (100)	20/20 (100)	
0	14/15 (93)	17/18 (94)	3/3 (100)	4/4 (100)		
1	4/14 (29)	16/21 (76)	3/3 (100)	3/4 (75)		
2	1/16 (6)	12/19 (63)	2/3 (67)	2/2 (100)		
3	5/11 (45)	11/18 (61)	5/6 (83)	5/5 (100)	30/30 (100)	28/30 (93)
4	4/11 (36)	14/23 (61)	1/2 (50)	4/4 (100)	21/30 (70)	25/30 (83)

Data are presented as number dead/total (% dead).
[a] Test 1; all other data is for test 2.

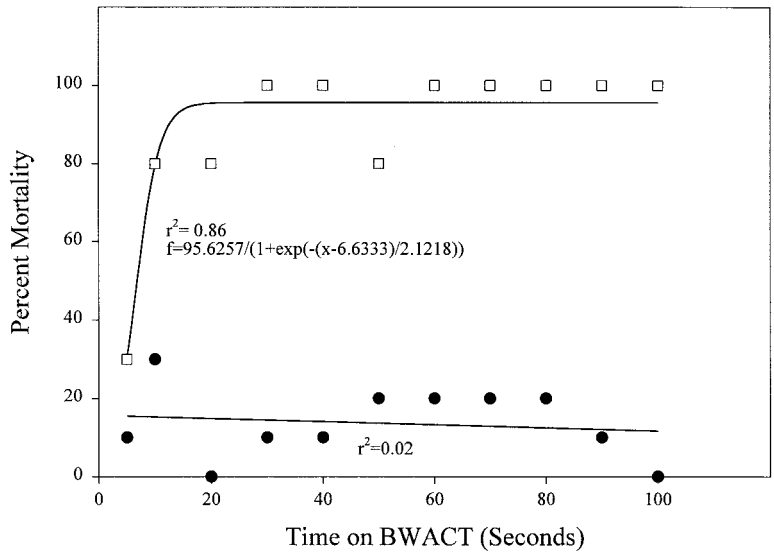

Fig. 7. Percentage of mortality of boll weevils as time spent on BWACTs increases. Squares, fresh tubes; circles, tubes in field for ~5 mo.

into the equation in Fig. 8, we would expect < 1% mortality solely as a result of forced-contact. In addition to low malathion adherence at initial forced-contact, we saw a consistent increase in malathion per weevil as time spent on a BWACT increased. Between 20 and 30 s of exposure, weevils had accumulated ≈1.5 μg, an amount at which can be expected to cause 100% mortality (Fig. 8). Malathion accumulation increased to 100 s, the longest time period we tested. Thus, even if no malathion adhered to weevils during initial forced-contact, they would still have accumulated sufficient malathion for high mortality if they remained on a BWACT for > 20 s. These findings run counter to those reported by Spurgeon et al. (1999) that forced-contact assays with BWACTs are not appropriate for assessing mortality. Their failure to kill naturally responding weevils may have been, as they suggested, a result of low levels of malathion in the BWACT coating (Figs. 7–9, solid circle). Our results indicate that the use of poor-quality BWACTs could produce the results they experienced. The relatively high mortality they reported for companion groups of

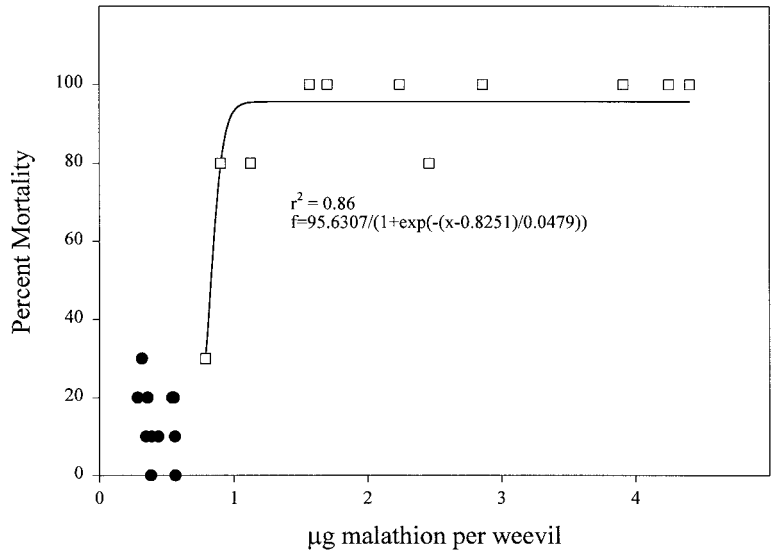

Fig. 8. Percentage of mortality of boll weevils as malathion accumulation increases. Squares, fresh tubes; circles, tubes in field for ~5 mo.

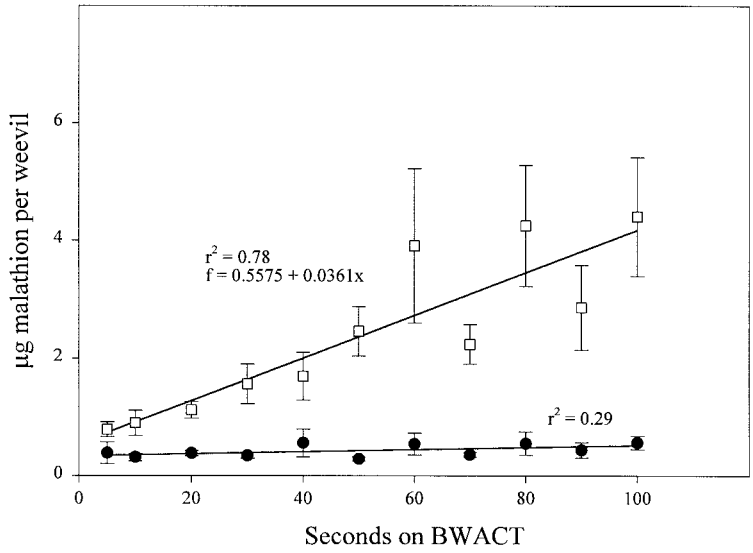

Fig. 9. Accumulation (with standard deviation) of malathion on individual boll weevils as time spent on BWACTs increases. Squares, fresh tubes; circles, tubes in field for ~5 mo.

weevils subjected to forced-contact assays may have resulted from a forced-contact assay that differed from ours.

In opposition to results with BWACTs in storage for 5 mo, we saw no increase in malathion adherence to weevils exposed to BWACTs aged in the field for 5 mo (Fig. 7). Weevils exposed to these field-aged BWACTs tended to accumulate between 0.25 and 0.5 μg each at all time periods (Fig. 9), and these amounts are not sufficient to cause high mortality (Fig. 8). Our data with the aged BWACTs suggest that such tubes may have small, scattered areas of malathion remaining on their surfaces, and although weevils may have contacted the spots, they were not able to accumulate $\geq$ 1 μg malathion, the amount required to produce mortality of or near 100% (Fig 8).

In summary, we make the following points. Properly conducted, the bioassay used to evaluate BWACT toxicity is exacting and time consuming, and results can vary depending on how strictly the procedure is followed. BWACTs with $>$ 30 ng of surface malathion per 1 μl of hexane consistently produced $\geq$ 90% mortality of weevils exposed to them in bioassays, but high mortality in bioassays was not always associated with correspondingly high levels of surface malathion. The total amount of malathion in and on BWACTs was not at all related to mortality. Similarly, no relationship emerged between total malathion and surface malathion. To replace the bioassay, we have described a simple chemical assay and presented an equation [1] with which boll weevil mortality can be conservatively predicted from results of the chemical assay.

Malathion accumulation on individual weevils increased linearly as time spent on fresh BWACT increased, but mortality increased sigmoidally as time increased and malathion accumulation increased. Malathion accumulations of $>$ 1.5 μg per weevil almost always resulted in 100% mortality. The amount of time required for a weevil to accumulate 1.5 μg will vary depending upon the amount of malathion present on the surface of a tube. Mortality of boll weevils placed on BWACTs in laboratory bioassays (forced-contact) was similar to that of those landing on tubes in the greenhouse after a short flight. Results of this study are being used to evaluate the quality of BWACT deployed by the Southeastern Boll Weevil Eradication Foundation (Montgomery, AL).

Acknowledgments

We thank Joe Stewart, Bill Kellum, and Debra Gary for technical assistance.

References Cited

Daxl, R., B. Ruiz Centeno, and J. Bustillo Caceres. 1995. Performance of the boll weevil attract and control tube (BWACT) in a 3-year area wide Nicaraguan boll weevil control program, pp. 933–997. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Fuchs, T. W., and R. Minzenmayer. 1992. Field evaluation of boll weevil bait sticks in West Texas, pp. 718–720. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Karner, M., and J. Goodson. 1993. Field evaluation of boll weevil bait sticks in Oklahoma, p. 1063–1065. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Karner, M., and J. Goodson. 1995. Performance of boll weevil attract and control tubes (BWACT) under Oklahoma conditions, pp. 1013–1016. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Langston, W. C. 1995. Boll weevil control with boll weevil attract and control tubes, 1994, p. 1012–1013. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Langston, W. C. 1996. Boll weevil control with boll weevil attract and control tubes, 1995, pp. 805–806. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

McGovern, W. L., J. W. Smith, E. J. Villavaso, and G. H. McKibben. 1993. Boll weevil suppression in Rutherford County, Tennessee with bait sticks, pp. 928–929. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

McGovern, W. L., E. J. Villavaso, and G. H. McKibben. 1995. Boll weevil bait stick test in Noxubee County, MS, pp. 928–928. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

McGovern, W. L., E. J. Villavaso, and G. H. McKibben. 1996. Final evaluation of 1994 boll weevil bait stick test in Noxubee County, MS, pp. 994–997. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

McKibben, G. H., J. W. Smith, and W. L. McGovern. 1990. Design of an attract-and-kill device for the boll weevil (Coleoptera: Curculionidae). J. Entomol. Sci. 25: 581–586.

McKibben, G. H., J. W. Smith, and E. J. Villavaso. 1991. Field research results on the boll weevil bait stick, pp. 622–623. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

McKibben, G. H., J. W. Smith, and E. J. Villavaso. 1993. Composition and Apparatus Useful for Attracting and Controlling Insect Pests. Serial no. 08/150,703. U.S. Patent Office, pending.

McKibben, G. H., R. G. Daxl, and J. W. Smith. 1994. Boll weevil bait stick use in Nicaragua in 1993, pp. 984–986. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Mulrooney, J. E. 2001. Enhancement of transfer of technical malathion from cotton leaves to boll weevils, Anthonomus grandis grandis Boheman, (Coleoptera: Curculionidae) using cottonseed oil, pp. 29–40. *In* G. H. McKibben [Ed]. The Use of Malathion ULV for Boll Weevil Eradication. Southwest. Entomol. Supplement No. 24.

Parker, R. D., D. A. Wolfenbarger, and B. A. Kniffen. 1995. Field evaluation of boll weevil attract-and-kill devices in the coastal bend of Texas, pp. 937–940. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Parvin, D. W. 1995. Test results of BWACT for early and midseason boll weevil control on commercial cotton in Mississippi and Tennessee, 1994, pp. 940–943. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Smith, J. W., E. J. Villavaso, G. H. McKibben, and W. L. McGovern. 1992. Boll weevil suppression using bait sticks in Tennessee, pp. 716–717. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Smith, J. W., W. L. McGovern, R. G. Jones, G. H. McKibben, and E. J. Villavaso. 1994. Late season use of boll weevil attract and kill devices, pp. 987–989. *In* Proceedings, Beltwide Cotton Production Res. Conference. National Cotton Council, Memphis, TN.

Spurgeon, D. W. 2001. Efficacy of field-aged bait sticks against the boll weevil. J. Cotton Sci. 5: 68–73. Online at http://www.jcotsci.org.

Spurgeon, D. W., J. R. Coppedge, J. R. Raulston, and H. Marshall. 1999. Mechanisms of boll weevil (Coleoptera: Curculionidae) bait stick activity relative to pheromone traps. J. Econ. Entomol. 92: 960–966.

Tumlinson, J. H., D. D. Hardee, R. C. Gueldner, A. C. Thompson, P. A. Hedin, and J. P. Minyard. 1969. Sex pheromones produced by male boll weevils: isolation, identification, and synthesis. Science 166: 1010–1012.

Villavaso, E. J., W. L. McGovern, and T. L. Wagner. 1998. Efficacy of bait sticks versus pheromone traps for removing boll weevils (Coleoptera: Curculionidae) from released populations. J. Econ. Entomol. 91: 637–640.

Received for publication 22 January 2002; accepted 29 July 2002.